# Photopheresis: Clinical Applications and Mechanism of Action

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**Photopheresis is a leukapheresis-based therapy that utilizes 8-methoxypsoralen and ultraviolet A irradiation. Photopheresis is currently available at approximately 150 medical centers worldwide. Recent evidence suggests that this therapy used as a single agent may significantly prolong life, as well as induce a 50%–75% response rate among individuals with advanced cutaneous T cell lymphoma (CTCL). Furthermore, a 20%–25% complete response rate with photopheresis alone, or in combination with other biologic response modifiers, has been obtained at our institution among patients with Sezary syndrome. These complete responses have been characterized by the complete disappearance of morphologically atypical cells from the skin and blood. The use of sensitive molecular techniques has also confirmed the sustained disappearance of the malignant T cell clone from the blood of patients with complete responses. In addition to the treatment of CTCL, numerous reports indicate that photopheresis is a potent agent in the therapy of acute allograft rejection among cardiac, lung, and renal transplant recipients. Chronic graft *versus* host disease also appears to be quite responsive to photopheresis therapy. Likewise, there may also be a potential role for photopheresis in the therapy of certain autoimmune diseases that are poorly responsive to conventional therapy. The immunologic basis for the responses of patients with these conditions is likely due to the induction of anticlonotypic immunity directed against pathogenic clones of T lymphocytes. Treatment-induced apoptotic death of pathogenic T cells and activation of antigen presenting cells are postulated to have important effects in this therapeutic process.** *Key words: photopheresis/transplantation/psoralen/lymphoma. Journal of Investigative Dermatology Symposium Proceedings 4:85–90, 1999*

Extracorporeal photopheresis is a leukapheresis-based immunomodulatory therapy that has been approved by the United States Food and Drug Administration since 1988 for the treatment of advanced cutaneous T cell lymphoma (CTCL). This therapy is presently available at approximately 150 treatment centers world-wide. At many institutions, photopheresis used with other biologic response modifiers has become a primary therapy for leukemic forms of CTCL, particularly for the Sezary syndrome that is characterized by erythroderma, circulating malignant T cells, and lymphadenopathy. Long-term follow up of patients who participated in the initial multicenter clinical trial conducted by Edelson *et al*, as well as follow up of our own patients, has indicated that photopheresis may be capable of producing significant prolongation of life in comparison with historic controls with a similar burden of disease (Edelson *et al*, 1987; Gottlieb *et al*, 1996). Moreover, the potential for cure of advanced forms of this malignancy was also realized during this trial as 25% of patients have experienced complete remission, with as many as 10% having no detectable residual disease for periods of up to 11 y.

In addition to therapy of CTCL, exciting recent results have indicated that photopheresis can be an effective means of reversing cases of resistant solid organ transplant rejection (Costanzo-Nordin *et al*, 1992a; Dall'Amico *et al*, 1995; Slovis *et al*, 1995; Wolfe *et al*, 1996; Barr *et al*, 1998). Similarly, a high response rate of chronic graft *versus* host disease to photopheresis also suggests the usefulness of this therapy in the post-bone marrow transplant setting (Greinix *et al*, 1999; Dall'Amico *et al*, 1997). Finally, the results of a randomized, controlled trial of the treatment of systemic sclerosis with photopheresis, as well as results of several earlier pilot studies that involved the therapy of pemphigus vulgaris and rheumatoid arthritis, also indicate the potential utility of photopheresis in the therapy of immunosuppressive drug resistant autoimmune disease (Rook *et al*, 1990, 1992; Malawista *et al*, 1991). In this review we will summarize the current use of photopheresis to treat cutaneous T cell lymphoma, allograft rejection, and autoimmune disease. Furthermore, the putative mechanisms of action will be discussed.

## CUTANEOUS T CELL LYMPHOMA (CTCL)

CTCL is a clonal lymphoid malignancy of helper T lymphocytes ($CD4^+$). Initial signs of this disease usually appear in the skin as plaques, tumors, or erythroderma, occasionally with concomitant lymphadenopathy and circulating malignant T cells. As the disease progresses, peripheral blood, nodal, and visceral involvement usually become more frequent and extensive. Although a variety of therapies directed at the cutaneous manifestations of CTCL, including electron beam radiation, topical mechlorethamine, as well as conventional psoralen and ultraviolet A photochemotherapy (PUVA), yield a high rate of initial clinical responses in regard to the skin disease (Thomsen *et al*, 1989; Vonderheid *et al*, 1989), these therapeutic regimens appear to produce little effect on extracutaneous disease. Furthermore, although multidrug chemotherapy has been recommended for advanced CTCL characterized by multiple tumors, erythroderma, or Sezary syndrome, it does not prolong survival and is associated with a substantial degree of morbidity (Kay *et al*, 1989). Thus, the initial multicenter trial of

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photopheresis conducted by Edelson *et al* that used this therapy on two successive days monthly for the treatment of erythodermic CTCL, yielded highly beneficial results with few, if any associated adverse effects (Edelson *et al*, 1987). Twenty-four of 29 (83%) erythrodermic patients, with disease resistant to a variety of conventional therapies, had improvement in their erythroderma with a mean time to development of a positive response within the skin of 22.4 wk. Moreover, nine patients (24%) experienced better than a 75% improvement in extent of their skin lesions, whereas 13 patients (35%) had a 50%–75% improvement in their skin lesions. Patients with the most significant degree of improvement had experienced noticeable clearing of their skin by the third or fourth month of treatment.

Emerging data from multiple treatment centers further substantiates a significant response rate achieved using photopheresis as monotherapy for patients with advanced CTCL. Gottlieb *et al* observed a 71% response rate, with a 25% complete response rate among 28 patients who received photopheresis monotherapy for at least 6 mo, the majority of whom had Sezary syndrome (Gottlieb *et al*, 1996). Furthermore, in a review of her recent clinical experience, Duvic *et al* reported a 50% overall response rate and an 18% complete response rate among 34 patients, 28 of whom had erythrodermic CTCL (Duvic *et al*, 1996). Thus, patients with Sezary syndrome or erythroderma appear to have frequent responses to photopheresis when this therapy is employed on at least two consective days every 4 wk.

In addition to improvement in the cutaneous manifestations of CTCL, a decrease in the extent of peripheral blood involvement has also been observed at different centers (Edelson *et al*, 1987; Duvic *et al*, 1996; Gottlieb *et al*, 1996). Among responders who have increased numbers of $CD4^+$ peripheral blood cells prior to therapy, the majority experienced a significant drop in the absolute numbers of these cells during their therapy. This decrease likely indicated a drop in the numbers of circulating malignant cells that bear CD4 on their surface. This conclusion is supported by the observation of Gottlieb *et al* that the numbers of atypical cells within the peripheral blood with a cerebriform nuclear morphology (Sezary cells) also decreased. Among the seven patients monitored by Gottlieb with Sezary syndrome who experienced complete responses, circulating Sezary cells became undetectable and T cell receptor gene rearrangement studies utilizing either Southern blot analysis or more sensitive polymerase chain reaction (PCR) assays have indicated the disappearance of the malignant clone from their peripheral blood in response to photopheresis therapy (Gottlieb *et al*, 1996; Lessin *et al*, 1991).

Long-term follow-up of erythrodermic patients who received photopheresis have suggested that this therapy may prolong survival beyond that expected with conventional therapies (Heald *et al*, 1992; Gottlieb *et al*, 1996). Gottlieb *et al* determined that survival of their patients exceeded 100 mo from the time of diagnosis of their disease, whereas previous studies of a comparable patient population, using established therapies other than photopheresis, have revealed survival rates of 30–40 mo (Sausville *et al*, 1988). A recent uncontrolled study failed to observe a statistically significant survival advantage of Sezary syndrome patients who received photopheresis as monotherapy (Fraser-Andrews *et al*, 1998). It is noteworthy, however, that many of the patients had received prior treatment with agents that clearly suppress the immune response, including cyclophosphamide, chlorambucil, fludaribine, and multidrug chemotherapy. Such pretreated patients have never responded to photopheresis monotherapy at our center. Typically, an immune adjuvant, such as interferon-$\alpha$ added to photopheresis, is necessary to induce a response in this setting (see below). Appropriate prospective controlled trials comparing photopheresis to other therapeutic agents for advanced CTCL, although difficult due with the paucity of patients with advanced disease, are still warranted to determine the full nature of the potential survival advantage produced by photopheresis.

During this period of extended observation, there have been few adverse effects of photopheresis recorded. These results are remarkable in view of recent data demonstrating not only a failure to prolong survival of CTCL patients with the use of intensive multidrug chemotherapeutic regimens combined with electron beam irradiation, but also in light of the high degree of morbidity of such treatment (Kay *et al*, 1989). Because of the paucity of adverse effects associated with photopheresis, as well as the potential for the prolongation of life, we frequently employ photopheresis as an initial therapy, along with other adjunctive biologic therapies including recombinant interferon-$\alpha$, for CTCL patients with peripheral blood involvement.

**Table I. Profile of CTCL photopheresis responders**

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| Presence of modest or small numbers of peripheral blood Sezary cells (10%–20% of mononuclear cells) |
| Short duration of disease (less than 2 y) |
| Normal or near normal numbers of cytotoxic T lymphocytes |
| Normal natural killer cell activity |
| No prior history of intensive chemotherapy |
| Absence of bulky lymphadenopathy or overt visceral disease |

**Table II. Potential indications for photopheresis**

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|---|
| Advanced cutaneous T cell lymphoma (FDA approved) |
| Allograft rejection |
| Graft *versus* host disease |
| Immunosuppressive drug resistant bullous disease |
| Systemic lupus erythematosus |
| Systemic sclerosis |

**Table III. Potential mechanisms of action of photopheresis**

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|---|
| Induction of T cell apoptosis |
| Macrophage/dendritic cell activation leading to enhanced processing of apoptotic T cell antigens and release of proinflammatory mediators |
| Induction of anticlonotypic immunity by the above processes |

Our experience during the last 13 y, and that of other investigators (Heald *et al*, 1992), has formed the basis for the development of a clinical profile of those patients most likely to respond to this therapy (**Table I**). We routinely recommend this treatment for patients who have either morphologic or Southern blot evidence of circulating malignant cells. Thus, patients with the Sezary syndrome or individuals with extensive plaque disease who have the presence of atypical peripheral blood cells observed on one micron-section analysis of buffy coat specimens, characterized by cerebriform nuclear morphology, are considered to be excellent candidates for photopheresis. In contrast, those without evidence of malignant peripheral blood cells as assessed using multiple parameters including one micron-section buffy coat analysis, gene rearrangement studies, or flow cytometry, have failed to respond at our institution to photopheresis monotherapy. For example, no patients with plaque disease covering greater than 10% of the skin surface area, or with erythroderma, in the absence of peripheral blood involvement, have responded to photopheresis. This is in contrast to patients with a similar degree of skin involvement in the presence of peripheral blood involvement, who have had substantial clinical improvement at our institution. Furthermore, those with an especially large tumor burden (white blood count $>20{,}000$ per $mm^3$, widespread bulky lymphadenopathy, visceral disease, or numerous cutaneous tumors) are unlikely to respond to photopheresis used as a single treatment modality. Although the authors are aware of several cases of tumor stage disease that have improved while undergoing photopheresis treatment, this form of CTCL is a good example of the need for concurrent therapy with electron beam irradiation with or without interferon-$\alpha$ (see below).

The overall integrity of the cell-mediated immune response is believed to be a critical factor for patient responsiveness to photopheresis. Our results indicate that normal levels of peripheral blood $CD8^+$ cytolytic T cells and natural killer cell activity are usually detected among patients with prominent clinical responses, whereas low levels of $CD8^+$ T cells and natural killer cell activity are typically measured among nonresponders. These results imply that $CD8^+$ cytolytic T cells and natural killer cells may be important components of the antitumor immune response. Moreover, these data may reflect the requirement for a greater degree of immune integrity so as to be able to generate

an immune response against the photo-altered malignant cells. These observations are highly relevant to the recent findings that the malignant clonal T cell population in CTCL may be susceptible to cell-mediated lysis by autologous cytotoxic T lymphocytes (Berger *et al*, 1996). Thus, strategies directed at the immunologic enhancement of cytotoxic T lymphocyte function through the use of biologic response modifiers may improve the efficacy of photopheresis (see below).

The use of immunosuppressive agents can exert a negative impact on the ability to generate an antitumor immune response. Thus, the extent of prior treatment with immunosuppresive chemotherapy negatively correlates with response to photopheresis therapy. (Previous immunosuppression in transplant patients experiencing active rejection and in marrow allograft recipients with graft *versus* host disease, however, does not appear to alter the response to photopheresis as described below.) In fact, studies using animal models of photopheresis have suggested that corticosteroids or immunosuppressive agents can inhibit the desired immunologic effects of this therapy, whereas granulocyte-macrophage colony stimulating factor (GMCSF) can enhance the response to photopheresis (Perez and Edelson, personal communication). Corticosteroids are known to inhibit host immune responses, including natural killer cell activity and production of GMCSF and tumor necrosis factor-$\alpha$ (TNF). Treatment-induced TNF, and perhaps GMCSF, may be important components of the antitumor response. We have recently determined that photopheresis induces the marked production of TNF and GMCSF by treated monocytes (Vowels *et al*, 1992; Rook, unpublished results). As a further example of photopheresis-induced immune responses that may be impaired by drugs, we attempt to avoid the use of prednisone in our CTCL patients. It is for reasons such as these that we advocate, whenever possible, the use of immune enhancing agents such as interferon-$\alpha$ rather than potentially immunosuppressive drugs for patients with CTCL.

For patients with disease parameters that place them in a poor prognostic category and that suggest a low probability of having a therapeutic response to photopheresis (extensive prior chemotherapy, large tumor burden, low numbers of peripheral blood $CD8^+$ T cells, depressed natural killer cell activity), adding certain adjunctive therapies in combination with photopheresis may improve the clinical course. We have obtained excellent results using interferon-$\alpha$ in low doses in combination with photopheresis. Patients are started on 2–2.5 million units subcutaneously three times weekly to minimize flu-like symptoms. Doses are gradually increased, as tolerated, to 5–7.5 million units four to five times weekly. The most typical regimen consists of 3–5 million units every other day. The majority of patients treated in this manner have experienced a significant drop in the numbers of circulating atypical cells in association with improvement in skin disease. In fact, a review of CTCL patients treated at our institution revealed that 11 of 14 patients experienced significant improvement following supplementation of their photopheresis regimen with interferon. Although the interferon is likely playing an exceedingly important role in these responses, it is the authors opinion that the two therapies appear to have an additive benefit over either alone.

Other biologic response modifiers that deserve exploration in combination with photopheresis include interferon-$\gamma$, interleukin-12 (IL-12), and GMCSF. Interferon-$\gamma$ and IL-12 exert substantial immune augmentatory effects on cytotoxic T lymphocyte function. Furthermore, the lymphocytes of patients with CTCL exhibit striking defects in the ability to produce these cytokines (Rook *et al*, 1993, 1995). A phase I trial using recombinant IL-12 alone for the treatment of CTCL has demonstrated a high clinical response rate and induction of cytotoxic T cell activity at sites of regressing lesions (Rook *et al*, 1999). Thus, if a vaccination effect of photopheresis with the subsequent generation of antitumor cytotoxic T cell responses is proposed as a primary mechanism of action (see below under mechanisms), then the concomitant use of IL-12 could potentially enhance this beneficial response. In addition, photopheresis is known to induce a high rate of apoptotic death of malignant T cells within the blood and it is presumed that antigens from these T cells are processed by the host immune response (Yoo *et al*, 1996). The administration of GMCSF following the photopheresis procedure might then increase the ability of antigen presenting cells to process antigenic components of cells undergoing apoptosis, leading to an augmented response to photopheresis. At least two patients at our center have had significant clinical improvement associated with the initiation of GMCSF therapy administered in a dose of 125 micrograms subcutaneously 1 h post-photopheresis.

Other adjuntive therapies used in combination with photopheresis include electron beam therapy that is frequently used at our institution as a debulking procedure for patients with large cutaneous tumors (Jones *et al*, 1995). Although there are anecdotal reports of patients with tumors who responded to photopheresis alone, in our experience concomitant electron beam therapy, often with interferon-$\alpha$, is usually required to produce tumor flattening. Use of methotrexate in doses of 15–25 mg weekly or azathioprine daily have also been suggested for patients with elevated white blood cell counts. Although these drugs may provide benefit by impeding the *in vivo* proliferation of the malignant T cells, one must also consider their potential for suppression of the host response to the photo-altered tumor cells induced by photopheresis. Finally, the use of other skin directed therapies, including topical mechlorethamine, topical carmustine, and PUVA therapy have all been found to be valuable adjuncts to clear the skin of patients who respond slowly to the combinations of agents described above.

## ALLOGRAFT REJECTION

As a novel alternative to the use of potent immunosuppressive drugs, striking results have been obtained by several groups using photopheresis to reverse acute rejection of heart allografts (**Table II**). Costanzo-Nordin *et al* at Loyola University were able to rapidly reverse eight of nine acute rejection episodes among seven cardiac allograft recipients using photopheresis (Costanzo-Nordin *et al*, 1992b). In most cases, one treatment, and no more than two photopheresis treatments, were capable of arresting the rejection episodes. Moreover, no serious adverse effects of treatment were observed among this patient population. In a subsequent randomized trial, this same group of investigators compared a single photopheresis treatment witth 3 d of high dose systemic corticosteroids in their ability to reverse cellular rejection of transplanted hearts (Costanzo-Nordin *et al*, 1992a). Following randomization and treatment of 16 patients, a comparable high rate of reversal of rejection occured within both groups with no significant differences being observed in response rate between the two groups.

In the experience of the authors, acute rejection should be treated aggressively at the outset with treatments on two consecutive days weekly for the first 2 wk and then on two consecutive days every other week until the rejection has resolved or improved significantly. Utilizing this regimen, eight of eight cardiac allograft recipients experiencing severe cellular rejection unresponsive to immunosuppressive drugs have had their rejection reversed at our institution (Macey *et al*, 1994; DeNofrio *et al*, 1999; unpublished results). Similarly, recent reports have indicated that intensive photopheresis therapy of refractory lung or renal allograft rejection can produce a high response rate with reversal of the acute rejection (Dall'Amico *et al*, 1997; Slovis *et al*, 1995; Wolfe *et al*, 1996).

Barr *et al* have also employed photopheresis on a prophylactic basis in an effort to prevent the manifestations of acute graft rejection among cardiac allograft recipients (Barr *et al*, 1998). They reported on a total of 61 patients, 27 of whom received standard triple drug therapy and 34 of whom received 24 photopheresis treatments during the first 6 mo post-transplantation in addition to standard therapy. Following 6 mo of treatment, the rate of acute rejection was significantly reduced among patients in the photopheresis group in comparison with the standard therapy group. Furthermore, those who received standard therapy had a significantly higher frequency of multiple rejection episodes. It is noteworthy that the incidence of infection was actually lower in the group receiving combined therapy. This latter observation likely reflects the diminished need for supplemental immunosuppressive therapy to treat acute rejection. Thus, the results of this study establish photopheresis as an important immunotherapy that can significantly decrease the frequency of rejection following cardiac transplantation.

Convincing new data has recently been presented suggesting that photopheresis can be a useful adjunctive therapy following allogeneic bone marrow transplantation to reverse graft *versus* host disease (GVH)

(Dall'Amico *et al*, 1998; Greinix *et al*, 1998). Greinix *et al* have reviewed the largest series including 21 patients, 15 of whom had chronic GVH and six of whom had acute GVH. An intensive treatment regimen comparable with that recommended for acute cardiac allograft rejection resulted in complete resolution of GVH in 12 of 15 with chronic disease and four of six with acute GVH, including normalization of hepatic dysfunction in seven of 10 cases (Greinix *et al*, 1998). These data strengthen the notion that controlled trials employing photopheresis to treat allograft rejection and GVH are urgently needed to unequivocally demonstrate the utility and safety of this therapy post-transplantation.

## AUTOIMMUNE DISEASE

Photopheresis has been used for a diverse group of autoimmune diseases with encouraging results (**Table II**). An initial study using photopheresis to treat autoimmune disease produced favorable results in patients with pemphigus vulgaris who were resistant to corticosteroids and immunosuppressive drugs (Rook *et al*, 1990). Four patients with uncontrolled disease, despite prolonged courses of treatment with high doses of prednisone in combination with cyclophosphamide or azathioprine, responded to photopheresis. All patients initially had improvement in the extent of their skin disease that allowed for significant tapering of other medications. Three of the four patients eventually experienced long-term remissions permitting discontinuation of all treatment. Significant reductions in serum levels of antiepidermal cell immunoglobulin G occured in conjunction with clinical improvement. Although relapses occured in all three, remission was easily reinduced following three to four additional monthly cycles of photopheresis. It is our experience that once clinical improvement occurs, gradual tapering of corticosteroids and immunosuppressive medications can proceed; however, simultaneous abrupt tapering of photopheresis along with the tapering of other medications may result in the early reoccurence of skin lesions. Photopheresis produced no serious adverse effects in any of the four patients during several years of follow up.

Recently, photopheresis has been successfully employed to treat systemic sclerosis (Rook *et al*, 1992). Substantial skepticism has arisen regarding the use of photopheresis for systemic sclerosis because it manifests primarily as a fibrosing disease with increased deposition of collagen within the skin and involved visceral organs. Despite its status as a fibrosing disease, recent observations have implicated the immune system as a prime factor in the genesis of the increased collagen production (Kahaleh and Roy, 1989; Kahari *et al*, 1990). Elevated serum levels of soluble interleukin-2 receptors in patients with active clinical disease support the association of T cell activation with disease progression. Furthermore, biopsy of involved tissues early in the evolution of the clinical disease has revealed tissue infiltration with activated helper T cells that may be releasing certain cytokines, particularly transforming growth factor β, which is a potent stimulator of collagen synthesis (Kulozik *et al*, 1990). Moreover, use of sensitive polymerase chain reaction technology has yielded results suggesting that a high proportion of patients with systemic sclerosis may have expanded clonal T cell populations in the peripheral blood, which may be responsible for mediating the fibrosing process (Lessin and Rook, unpublished observations).

The results of a multicenter randomized, single-blinded trial to examine the efficacy and safety of photopheresis in the reversal and prevention of progression of skin disease in systemic sclerosis of recent onset and rapid development, have recently been published (Rook *et al*, 1992). Seventy-nine patients with systemic sclerosis of recent onset (mean duration of symptoms was 1.83 y) and progressive skin involvement entered a randomized, parallel group clinical trial comparing photopheresis given on two consecutive days every 4 wk to treatment with D-penicillamine. Skin severity scores (skin hardening), percentage skin surface area involvement, hand closure, and oral aperture were evaluated monthly by blinded examiners. A variety of other evaluations including pulmonary function tests, skin biopsies, and serologies were obtained at baseline and after 6 and 12 mo of treatment. During this trial 56 patients received 6 mo of therapy (31 received photopheresis), whereas 47 received 10 mo of therapy (29 on photopheresis). By 6 mo of treatment, 21 of 31 (68%) patients who received photopheresis had experienced significant softening of the skin, in comparison with eight of 25 (32%) who received D-penicillamine. It is noteworthy that whereas only three of 31 (10%) of those who received photopheresis had experienced significant worsening of their skin severity score after 6 mo of treatment, eight of 25 (32%) who received D-penicillamine had significant worsening. Thus, in the early phases of treatment, a significantly higher response rate was obtained with photopheresis ($p = 0.02$). At both the 6 and 10 mo evaluation point, the mean skin severity score, mean percentage involvement, and mean oral aperture measurements were significantly improved from baseline among those who received photopheresis. Mean right- and left-hand closure measurements had also improved significantly by 10 mo of therapy. Skin biopsy studies demonstrated an association between clinical improvement and decreased thickness of the dermal layer.

It is noteworthy that adverse effects of photopheresis were minimal during this trial and did not require discontinuation of treatment by any of the patients. In contrast, 25% of patients who received D-penicillamine were required to permanently discontinue this drug due to side-effects or rapid progression of disease while on this therapy. Thus, photopheresis appears to produce early improvement with few side-effects when used for aggressive cases of recent onset systemic sclerosis.

In addition to studies involving pemphigus and systemic sclerosis, the results of pilot studies have suggested the potential efficacy of photopheresis for rheumatoid arthritis (Malawista *et al*, 1991), epidermolysis bullosa acquisita (Miller *et al*, 1995; Gordon *et al*, 1997), atopic dermatitis (Prinz *et al*, 1994), and systemic lupus erythematosus (Knobler *et al*, 1992). Other clinical indications that have been studied where efficacy has not been demonstrated include multiple sclerosis, chronic hepatitis C, and AIDS-related complex.

## ADVERSE EFFECTS

The general experience since 1985 is that photopheresis has been extremely well tolerated. The most common adverse effect has been the sporadic occurence of psoralen-induced nausea. This is usually mild with a short duration of 30–60 min. It occasionally can be associated with vomiting and diaphoresis. If nausea is recurrent, the dosing can be staggered. For example, each 10 mg capsule can be administered every 10 min. This split dose regimen typically produces satisfactory blood levels within 2 h after the last capsule, yet nausea often can be eliminated. The recent FDA approval of a new liquid form of psoralen that can be mixed directly with the leukocytes within the UVAR device, which will permit the precise pharmacologic regulation of 8-MOP levels, will obviate the need for oral administration of 8-MOP in the future (Knobler *et al*, 1993).

Hypotension occurs uncommonly during the leukapheresis phase, particularly among those taking antihypertensive agents or diuretics. These medications are often held until the conclusion of the treatment. As an alternative, small volumes of normal saline may be infused just prior to the initiation of the treatment as a preventive measure. In most cases, patients with advanced cardiomyopathy and aortic stenosis have tolerated photopheresis without difficulty. These observations indicate that substantial homeostatic derangements of vascular volume are often required for the development of hypotension.

Low grade fevers occuring 4–12 h after reinfusion of the treated cells are common during the early phases of therapy in patients with CTCL. Patients are usually asymptomatic and antipyretics are not needed. Fever appears to be unrelated to bacteremia and is associated with the most marked clinical responses to treatment. Recent observations indicate that photopheresis is responsible for the induction of proinflammatory and pyrogenic cytokines from monocytes (see below). This immunologic effect is the likely cause for the post-treatment febrile response. Nevertheless, because this therapy involves venous cannulation of the frequently immunosuppressed patient, it is recommended that blood cultures be obtained and that all potential sources of infection be evaluated.

Although normal leukocytes are exposed to psoralen and ultraviolet during the photopheresis procedure itself, depletion of these blood elements has not been noted. Furthermore, clinical evidence of photopheresis-induced immunosuppresion such as the development of neoplasia or opportunistic infections, has not been observed. Thus the fact that photopheresis produces minimal adverse effects and can provide substantial benefit indicates that this therapy has clear advantages in comparison with chemotherapeutic and immunosuppressive agents.

## MECHANISM OF ACTION OF PHOTOPHERESIS

Substantial new information has emerged that suggests that photopheresis exerts its clinical effects via several mechanisms of action (**Table III**). The biologic effects of psoralens are critical to the treatment process. 8-MOP rapidly diffuses into nucleated cells and upon exposure to ultraviolet A irradiation, covalent cross-linking of DNA occurs that ultimately results in the proliferative arrest of treated cells (Song and Tapley, 1979). Furthermore, the combination of 8-MOP and ultraviolet A irradiation causes the majority of treated T cells to undergo apoptosis within 48 h of the photopheresis procedure (Yoo *et al*, 1996). Because large or activated T cells may be particularly sensitive to the antiproliferative effects of 8-MOP and ultraviolet A, such T cells within the peripheral blood of patients with autoimmune disease or allograft rejection may be especially susceptible targets for psoralen and ultraviolet A-mediated damage.

Although a high rate of apoptosis of treated T cells occurs after exposure to at least 50 ng 8-MOP per ml and 2 J per $cm^2$ of ultraviolet A irradiation, peripheral blood macrophages appear to be comparatively resistant to the apoptotic effects of 8-MOP and ultraviolet A (Yoo *et al*, 1996). Moreover, upon completion of the photopheresis procedure, treated monocytes appear to have an activated phenotype with enhanced expression of proinflammatory cytokines, adhesion molecules, and major histocompatibility proteins that are essential for antigen presentation (Vowels *et al*, 1992; Moor *et al*, 1995). In fact, flow cytometry studies have revealed that the critical accessory molecule CD86 and the adhesion molecule CD36 are routinely and rapidly upregulated on the surface of macrophages upon completion of photopheresis (Fimiani *et al*, 1997; Kao and Rook, unpublished observations). These findings are undoubtedly relevant to the observation that photopheresis-treated macrophages exhibit a significantly increased ability to phagocytose apoptotic T cells (Yoo *et al*, 1996). Thus, one could invoke the enhanced uptake, processing, and presentation by macrophages and dendritic cells of apoptotic T cell antigens from dominant clones of pathogenic T cells as a possible scenario for the induction of anticlonotypic immunity by photopheresis (Albert *et al*, 1998).

Evidence that photopheresis induces anticlonotypic immunity has been suggested by recent observations using a number of different experimental animal models. Study of the murine model of experimental allergic encephalitis has been especially revealing in this regard (Khavari *et al*, 1988). In this model, rats injected with myelin basic protein develop a paralytic illness associated with T cell destruction of the nervous system. The pathogenic T cells that mediate this destruction can be isolated and cloned *in vitro*. When naïve syngeneic rats are inoculated with the cloned T cells, all of the features of experimental allergic encephalitis are reproduced. If the pathogenic clones are first treated with psoralen and ultraviolet A and then infused, however, the animals are protected from the development of disease upon subsequent challenge with the pathogenic T cells. Protection from disease appears to be mediated by the generation of clone-specific suppressor T cells that have developed in response to the psoralen and ultraviolet A-modified pathogenic cells. Perez *et al*, employing a model of cutaneous allograft rejection, have similarly obtained evidence of stimulation of an antigen-specific suppressor T cell response when alloreactive effector T cells are treated with psoralen and ultraviolet A and infused into syngeneic animals (Perez *et al*, 1989). These results indicate that, at least in the setting of these animal models, an active immunization process can occur following the administration of photoinactivated syngeneic T cell clones.

Because of its efficacy in the treatment of diseases of immune activation, concern has been expressed regarding the potential immunosuppressive effects of photopheresis. Studies in our laboratory have indicated that photopheresis does not produce measurable suppression of T cell responses against recall protein antigens, such as tetanus toxoid, nor does it suppress primary immune responses against *de novo* protein antigens such as Keyhole Limpet hemocyanin (Suchin *et al*, 1999). Furthermore, a panel of delayed type hypersensitivity skin tests placed on seven patients with systemic sclerosis remained unaltered following 6 mo of photopheresis therapy. It is possible, however, that photopheresis induces specific suppression of responses against alloantigens, as suggested by the observations of Perez *et al* (1989) using a murine model of cutaneous allografting. These latter observations are particularly relevant to the early clinical findings that photopheresis can reverse rejection of transplanted hearts. Nevertheless, it should be emphasized that significant clinical evidence of photopheresis-induced immunosuppression, such as the development of neoplasia or opportunistic infections, has not been observed. Moreover, when used in the already heavily immunosuppressed populations that have received solid organ or bone marrow allografts, an increased frequency of bacterial infections has not been observed. Thus, the fact that photopheresis produces minimal adverse effects and provides substantial benefit for a number of immune mediated diseases, indicates that this therapy has clear cut advantages in comparison with chemotherapeutic and immunosuppressive agents in the treatment of these conditions.

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